JOURNAL OF CLINICAL MICROBIOLOGY, Dec. 2000, p. 4425–4429 Vol. 38, No. 12
0095-1137/00/$04.00+0

Prevalence of Diarrheagenic *Escherichia coli* in Finns with or without Diarrhea during a Round-the-World Trip

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Received 5 July 2000/Returned for modification 31 August 2000/Accepted 24 September 2000

The incidence of diarrhea and the prevalence of bacterial enteropathogens, viruses, and parasites in feces of subjects with and without diarrhea were evaluated in 204 Finns traveling round the world (from Finland to China, Malaysia, Australia, Fiji, Chile, and Brazil and back to Finland). Special emphasis was placed on the finding of diarrheagenic *Escherichia coli* (enterotoxigenic, enteropathogenic, Shiga toxin-producing, and enteroaggregative strains) by PCR from growth on primary culture plates. From the PCR-positive samples, corresponding strains were isolated, confirmed as *E. coli*, and O serotyped. Of all the subjects, 37% experienced a total of 90 episodes of diarrhea. No adenoviruses or rotaviruses were detected, and findings of parasites were insignificant. In contrast, enteropathogenic bacteria were present in 62% of the 65 diarrheal and in 33% of the 127 nondiarrheal samples ($P < 0.001$); diarrheagenic *E. coli* strains were found in 35 and 26% of these, respectively (not statistically significant). As a single pathogen, *E. coli* was found in 20 and 24% of samples (not significant). Of all diarrheagenic *E. coli* strains, enteropathogenic strains were the most commonly found independently of the clinical picture of the subjects, whereas *Salmonella enterica* as a single pathogen was the most common non-*E. coli* organism found in diarrheal samples. Multiple bacterial pathogens were found 10 times more commonly in diarrheal than in nondiarrheal samples (20 versus 2%; $P < 0.001$).

Each year, millions of people traveling from industrialized countries to developing countries experience traveler's diarrhea (TD). TD is commonly associated with symptoms such as abdominal pain or cramps, nausea, vomiting, and fever (26). Although the disease is usually self-limiting, it may ruin holidays and cause great expense to the traveler. Contaminated food and water are the most important vehicles for the transmission of TD (3).

Bacteria are the most common causative agents of TD. Of these, enterotoxigenic *Escherichia coli* (ETEC) is generally believed to be the major cause of TD, irrespective of the locality (16). An oral cholera vaccine containing killed *Vibrio cholerae* and purified cholera toxin B subunit has been reported to provide some protection against TD due to ETEC (18). However, the role of other *E. coli* pathogroups, such as Shiga toxin-producing *E. coli* (STEC), enteropathogenic *E. coli* (EPEC), and enteroaggregative *E. coli* (EAEC), in TD has been overlooked because of limited detection methods.

Diarrheagenic *E. coli* strains were among the first pathogens for which molecular diagnostic methods were developed. Molecular methods, especially PCR, are currently considered the most reliable techniques for differentiating diarrheagenic *E. coli* strains from nonpathogenic members of the stool flora and for distinguishing one *E. coli* pathogroup from another (16). This has made it possible to reevaluate the role of various diarrheagenic *E. coli* groups in TD. In this study, we used PCR to detect diarrheagenic *E. coli* (ETEC, EPEC, STEC, and EAEC) from stool cultures; i.e., all *E. coli* organisms found were actually alive and therefore potentially capable of causing symptoms. Additionally, since a new ETEC–recombinant B-subunit oral vaccine was been developed, we wanted to test whether it provide protection against TD.

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MATERIALS AND METHODS

Setting. The Ethical Committee of the Finnish National Public Health Institute approved the study protocol. A charter flight on an aircraft with 320 seats had been booked for a whole journey round the world. An introductory letter was sent via the travel agent to all the tour participants. The study was carried out among those 204 travelers who agreed to participate. They traveled round the world (Helsinki, Finland [19 April], Shanghai, China [20 to 22 April], Kuching, Malaysia [22 to 24 April], Sydney, Australia [24 to 27 April], Fiji [27 to 30 April], Santiago de Chile, Chile [30 April to 2 May], Recife, Brazil [2 to 4 May], and Helsinki, Finland [5 May]) within 16 days in spring 1996. Each volunteer received two doses of the ETEC–recombinant B-subunit oral vaccine (SBL Vaccine Ab, Stockholm, Sweden) (10, 22) or a placebo containing heat-killed *E. coli* K-12 suspended in phosphate-buffered saline. The first dose was to be ingested about 21 days before the trip, and the second dose was to be ingested 2 weeks after the first dose. All participants gave at least two fresh fecal samples, one each immediately before and after the trip. In addition, if diarrhea occurred during the journey, a fecal sample was collected as soon as possible and another sample was collected on the following day. Also, if diarrhea occurred within a week after the return home, a sample was taken. Additionally, a sample for parasitology was taken 4 weeks after return.

Definitions. A diarrheal episode was defined as diarrhea, reported by the patient, with at least three unformed stools in an 8-h period, with nausea, vomiting, abdominal pain, or cramps. At least two symptomless days between two episodes and different findings in bacterial culture were required to qualify the episodes as separate.

Culturing and identification of bacteria. During the trip, a sample from each diarrheal episode was cultured as soon as possible on a cystine–lactose–electrolyte-deficient (CLED) agar plate for *E. coli* and other gram-negative bacteria by two specially trained nurses who participated in the trip. The plates were incubated overnight at 35°C in a small incubator (Thermocult; Boehringer, Mannheim, Germany). All samples were also preserved in two transport tubes (Transpocult [Orion Diagnostica, Espo., Finland] and Probact [Technical Service Consultants Ltd., Bury, United Kingdom]) and stored in a cool container (insulated transport box RCW2; Electrolux, Vianden, Luxembourg) for further analyses in Finland. The samples taken in Finland were processed immediately at the Laboratory of Enteric Pathogens of the Finnish National Public Health Institute. After the trip, the cultures on CLED plates were analyzed for different diarrheagenic *E. coli* pathogroups by the PCR method (see below). For isolation of *Salmonella*, *Shigella*, *Campylobacter*, *Yersinia*, *Aeromonas*, and *Plesiomonas* species, the following agar plates were used: xylose-lysine-desoxycholate, Önöz (E. Merck, Darmstadt, Germany), campylobacter blood-free selective (modified

TABLE 1. Prevalence of TD among 204 subjects traveling round the world and numbers of diarrheal stool samples available for bacterial culture

Parameter	No. (%)		
	Vaccinees (n = 101)	Controls (n = 103)	Total (n = 204)
No. of subjects with TD	31 (31)[a]	44 (43)	75 (37)
No. of TD episodes (≥1 in a patient)	38 (38)[a]	52 (50)	90 (44)
TD and diarrheal stool sample available			
No. of patients	24	36	60
No. of episodes	27	38	65

[a] Not significantly different from control value (Fisher's exact test).

CCDA-Preston; Oxoid, London, United Kingdom), cefsulodin-Irgasan-novobiocin, and aeromonas selective (Difco Laboratories, West Molesey, United Kingdom). In addition, selenite F broth was used to enrich salmonellae. Plates and selenite broth were incubated at 35°C for 18 h, except cefsulodin-Irgasan-novobiocin plates, which were incubated at 30°C for 24 h, and the campylobacter plates, which were incubated in jars in an atmosphere containing 5% O_2 and 10% CO_2 at 42°C for 48 h. The identification of all suspected isolates was carried out by standard methods (15). Of the pretrip cultures, 50 randomly chosen cultures were analyzed for bacterial pathogens as described above. If a positive PCR result was obtained, the pretrip culture of the subject was checked for that particular gene.

From each PCR-positive fecal culture, distinct *E. coli*-like and other gram-negative colonies were isolated and tested for the presence of the sequence initially identified as a positive result. As many colonies as required (but no more than 60) to find the isolate carrying these particular genes were assayed. The isolates were characterized biochemically by Api 20 E (bioMerieux SA, Marcy l'Etoile, France). *E. coli* strains that carried *eaeA* and were negative for *stx* genes were identified as EPEC (11), those that were EAEC PCR (23) positive were identified as EAEC, those that were positive for heat-labile enterotoxin (LT) or heat-stable enterotoxin (ST) were identified as ETEC, and those that were *stx* positive were identified as STEC.

Virology and parasitology. The investigation for rotaviral and adenoviral antigens in samples from diarrheal patients was carried out during the trip by latex agglutination (Diarlex Rota-Adeno kit; Orion Diagnostica) according to the manufacturer's instructions. The specimens for parasitology were collected before the trip and 4 weeks after return and analyzed at the Unit of Parasitology of HUCH-Laboratory Diagnostics, Helsinki, Finland. The standard formalin-ether concentration method was used (15).

PCR. Detection of genes encoding LT (17) or ST (5) of ETEC and Stx1 or Stx2 toxins of STEC (17), genes specific for EAEC (23), and *eaeA* (7, 13) gene sequences indicative of EPEC was carried out by PCR from the primary fecal cultures on CLED plates. Prior to PCR amplification, a loopful of gram-negative bacterial growth was taken from the first streaking area of the culture. It was suspended in 0.5 ml of sterile distilled water and was boiled for 20 min. *E. coli* strains ATCC 35401 (LT^+ ST^+), ATCC 43886 (LT^+), ATCC 43894 (stx_1^+ stx_2^+ $eaeA^+$), and RH 4260 (*E. coli* 17-2, EAEC) (1) were used as positive controls in each batch of PCR. The negative control was sterile distilled water.

Serotyping. O grouping of *E. coli* was carried out by bacterial agglutination with antisera against EPEC (O26, O44, O55, O86, O111, O112, O114, O119, O124, O125, O126, O127, O128, and O142) and other O groups (O1, O2, O4, O6, O7, O8, O9, O11, O15, O16, O18, O22, O25, O50, O75, O77, O83, O85, O86, O100, and O157) as previously described (24). *Salmonella* isolates were serotyped and identified by standard methods (12, 19).

Statistical methods. Differences between the numbers of microbial findings from travelers with and without diarrhea were compared by using Fisher's exact test (Epi-Info 6.04b [World Health Organization, Geneva, Switzerland] and CDC [Atlanta, Ga.]). A P value of <0.05 indicated statistical significance.

RESULTS

Of the 204 subjects, 105 were female. The age range of the subjects was 17 to 86 years (mean, 64 years). Seventy-five subjects (37%) experienced a total of 90 episodes of diarrhea (Table 1). The number of diarrheal cases and the distribution of bacterial findings did not differ statistically significantly between the vaccinees (n = 101) and the controls (n = 103). Therefore, the data on these groups are pooled in the data presented below.

Diarrheal episodes took place in two peaks; the first occurred 2 days and the second occurred 18 days after departure. This second peak appeared 1 day after the return to Finland (Fig. 1). A stool sample was available for culture from 65 episodes of diarrhea (60 subjects; 80% of all 75 patients with diarrhea and 72% of all 90 diarrheal episodes) (Table 1; Fig. 1). The causes of the diarrheal episodes in China and Malaysia remained mostly unknown. The episodes in the latter part of and after the trip probably originated in South America.

Enteropathogenic bacteria were found in 62% of the 65 diarrheal episodes (Table 2). The corresponding value for the 127 subjects without diarrhea was 33% ($P < 0.001$). No rotaviral or adenoviral antigens were detected in diarrheal patients. Pathogenic *E. coli* was found in 35% of samples from the 65 diarrheal episodes (n = 23) and in 26% of samples from healthy returnees (n = 33) (not statistically significant). Samples from 13 diarrheal episodes yielded a single pathogen, and samples from 10 yielded a mixed culture with some other bacterial pathogen (Table 2). The numbers of single *E. coli* findings in samples from diarrheal episodes and from healthy returnees did not differ statistically. Non-ETEC strains were found in samples from 29% (n = 19) of the episodes: *eaeA*-positive EPEC was found in 26% (n = 17) and was accompanied by EAEC in 8% (n = 5). The ST-encoding gene of ETEC was found in samples from three of the diarrheal episodes, and the LT gene was found in one. All of the four episodes (6%) caused by ETEC started during or after a stay in South America (Fig. 1). Because the number of ETEC strains identified was low, the efficacy of the vaccine could not be evaluated. Strains of various *Salmonella* serotypes were the most common non-*E. coli* pathogens, and they were isolated either alone or with another bacterial pathogen in samples from 24% (n = 14) of the diarrheal episodes and 3% (n = 4) of the healthy returnees (P = 0.003). The numbers of single non-*E. coli* organisms in diarrheal episodes and in healthy returnees differed significantly (22 versus 7%; P = 0.003).

Several bacterial pathogens were detected in 20% (n = 13) of the diarrheal episodes, whereas in specimens from 127 healthy subjects several pathogens were found on return in 2% (n = 3, P = < 0.001). In these three latter cases, all organisms were EPEC in combination with either an *Aeromonas* sp., *Salmonella*, or STEC. Of all bacteria found, the EPEC strains were the most common, independent of the clinical status of the subjects, being detected in a total of 39 samples, whereas *Salmonella enterica* was more commonly found as a single pathogen in samples from subjects with diarrheal episodes than in samples from healthy returnees (12 versus 2%, P = 0.005). All except three (those with EAEC, STEC stx_2, or an *Aeromonas* sp.) of the pretrip samples from 92 subjects were negative for pathogenic *E. coli* and conventional enteric pathogens (Table 2).

Specific *E. coli* strains were isolated from the stool cultures that were positive for diarrheal *E. coli* in PCR. All were O nontypeable in serotyping with the 35 sera used. Four subjects carried the same parasites before and after the trip (one had *Entamoeba coli* and *Endolimax nana*, two had *Entamoeba coli*, and one had *Giardia lamblia*). One of them had diarrhea after the trip. Three subjects carried *Endolimax nana* 4 weeks after return but had no diarrhea during or after the trip.

DISCUSSION

In this prospective study, a group of 204 Finns traveled round the world in 16 days. The incidence of diarrhea was registered, and a stool sample was taken and cultured immediately during the course of the journey for bacterial and viral

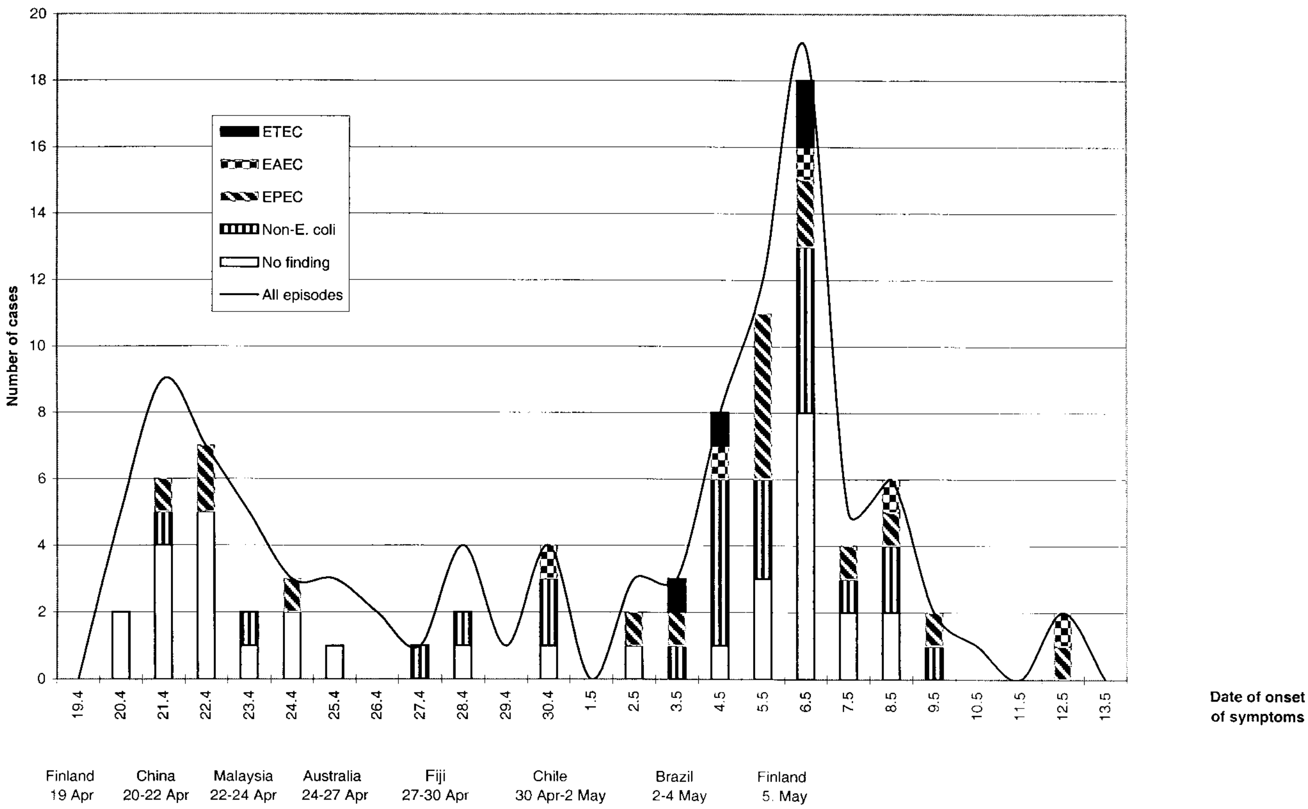

FIG. 1. Onset and etiology of diarrheal episodes during and after a round-the-world trip. Dates are given in the form day.month.

agents by a nurse or laboratory technician who traveled with the group. The samples were also preserved in transport tubes for further studies. The study protocol procedures were similar to those of our previous study (18); additionally, however, new, more specific molecular methods were used to test for the diarrheagenic groups ETEC, EPEC, STEC, and EAEC.

The episodes of diarrhea seemed to occur in two phases: during the first days after the departure and in the last days before leaving for home and after homecoming. The number of diarrheal samples available for bacterial investigations followed evenly the curve of diarrheal episodes, suggesting that the findings of the study were not biased by any uneven availability of the samples. The overall proportion of samples from the returning healthy subjects that were positive for bacteria was statistically significantly lower than that in subjects with diarrhea. The causes of diarrhea in the beginning of the trip in China and Malaysia remained mostly unknown. At least some of these episodes may have been functional, caused by the stress of the tight schedule of the trip in elderly subjects (mean, 64 years).

Pathogenic *E. coli* were found in 35% of the samples from 65 episodes of diarrhea and almost equally often (26%) in the subjects without diarrhea. In addition, as a single pathogen, various *E. coli* strains were even slightly more common in subjects without diarrhea (found in 24%) than in subjects with diarrheal episodes (found in 20%), suggesting that their enterovirulence is not high compared with that of, for example, *S. enterica*. However, multiple pathogens were statistically significantly more commonly detected in samples from diarrheal episodes (20%) than in samples from nonsymptomatic returnees (2%). A similarly high number of multiple pathogens causing TD was also found in our previous study on TD among Finns (14). This finding of multiple pathogens may indicate that the colonization by *E. coli*, especially by EAEC or EPEC, may provide a second pathogen with better conditions for invading the intestine and causing diarrhea.

ETEC has been regarded as the most common pathogen in TD, irrespective of the tourist destination. The percentages of diarrheal episodes caused by ETEC, however, have varied from study to study, from nation to nation, and from season to season (3, 9, 14). In the present study, ETEC strains were found in samples from only 4 (6%) of the 65 diarrheal episodes. The ST gene of ETEC was found in three patients, and the LT gene was found in in one. A previous Swedish TD study showed that ETEC infections were commonly acquired by tourists visiting Asia, Africa, and South America (9). In our study, the subjects traveled round the world and spent time in six countries and on three continents (Asia, Australia, and South America). However, ETEC strains detected in samples from four subjects with diarrhea were from episodes which started after a stay for 2 to 3 days in countries of South America. Thus, all eight ETEC strains detected probably originated in South America. The small number of ETEC strains identified did not allow much chance for vaccine efficacy calculations. Nevertheless, ETEC has been reported to be a common diarrheal pathogen in Brazil (28). In the present study, although the number of TD episodes was higher after a visit to South America than after earlier visits to other places, EPEC rather than ETEC was the major pathogroup of *E. coli* found in these diarrheal episodes.

The *eaeA*-positive EPEC strains were the most commonly found in both diarrheal samples and samples taken from healthy subjects on return. None of the EPEC strains isolated belonged to the classic EPEC O serogroups (20). This may

TABLE 2. Bacterial findings in stool samples from 65 diarrheal episodes of subjects traveling round the world and from 127 healthy subjects on the same trip on return

Finding[a]	No. (%) of samples with finding		P[b]
	Diarrheal episodes (n = 65)	Healthy returnees (n = 127)	
Any pathogen	40 (62)	42 (33)	<0.001
Single pathogens			
E. coli[c]	13 (20)	30 (24)	NS
EPEC *eaeA*	10 (15)	19 (15)	NS
EAEC	1 (2)	6 (5)	NS
ETEC (ST positive)	1 (2)	2 (2)	NS
ETEC (LT positive)	1 (2)	2 (2)	NS
STEC stx_2	0	1 (1)	NS
Other bacteria	14 (22)	9 (7)	0.003
Salmonella enterica[d]	8 (12)	3 (2)	0.005
Campylobacter jejuni	2 (3)	0	NS
Aeromonas sp.	3 (5)	6 (5)	NS
Plesiomonas shigelloides	1 (2)	0	NS
Multiple pathogens	13 (20)	3 (2)	<0.001
EPEC *eaeA* + EAEC	3 (5)	0	0.015
EPEC *eaeA* + STEC stx_2	0	1 (1)	NS
EPEC *eaeA* + *Aeromonas* sp.	2 (3)	1 (1)	NS
EPEC *eaeA* + *S. enterica* serotype Give	1 (2)	0	NS
EPEC *eaeA* + *S. enterica* serotype Infantis	1 (2)	0	NS
EPEC *eaeA* + *S. enterica* serotype Havana	0	1 (1)	NS
ETEC (ST) + *Aeromonas* sp.	1 (2)	0	NS
ETEC (ST) + *S. enterica* serotype Javiana + *S. enterica* serotype Havana	1 (2)	0	NS
EAEC + *S. enterica* serotype Havana + *Aeromonas* sp.	1 (2)	0	NS
S. enterica serotype Anatum + *Aeromonas* sp.	1 (2)	0	NS
S. enterica serotype Give + *Aeromonas* sp.	1 (2)	0	NS
Shigella flexneri + *Yersinia enterocolitica*	1 (2)	0	NS

[a] Of the pretrip samples from 92 healthy subjects, one was positive for EAEC, one was positive for STEC stx_2, and one was positive for an *Aeromonas* sp.
[b] Fisher's exact test. NS, not significant.
[c] Of the eight ETEC isolates found, two were from vaccinees and six were from controls (not significant).
[d] *S. enterica* serotypes: Havana (one patient), Hadar (two patients), Javiana (two patients), Hadar and Javiana (one patient), Uganda (one patient), and 9,12:−:− (one patient).

mean that the strains isolated represent a new, probably less virulent, EPEC group or that the old classification of "dyspepsiekoli" is not valid any more, as suggested recently by Sunabe and Honma (27), who studied the relation between the O serogroup and pathogenic factor genes in *E. coli*. In developing countries, EPEC is still an important cause of infant diarrhea but it has not been traditionally implicated as a cause of TD (16). However, in a previous study, EPEC was identified by phenotypic methods in 3 to 7% of people traveling to Morocco and suffering from TD (14). In Brazil, one of the countries visited during the present trip, EPEC has been the most prevalent enteropathogen in diarrheal children (21). In our present study, the finding that EPEC was the most common *E. coli* pathogroup in stools of diarrheal patients suggests that it, either alone (15%) or with another pathogen (9%), is able to cause TD, although nonsymptomatic carriage of EPEC as a single bacterial pathogen was also common (15%).

EAEC has been implicated as an etiological agent of diarrhea in developing countries and in outbreaks of gastroenteritis in industrialized countries (25). Very little is known about its significance in TD, since only a limited number of studies have been published. In the present study, only one subject with TD had EAEC as a single pathogen. It was, nevertheless, a common finding in subjects both with (8%) and without (5%) diarrhea. In Brazil, EAEC is a common cause of persistent diarrhea in children (4). In Japan in 1993, EAEC caused a massive outbreak of gastrointestinal illness in almost 2,700 schoolchildren (8). In a previous TD study, EAEC was isolated from 6 and 4% of U.S. military personnel with diarrhea who participated in shipboard exercises in South America and West Africa, respectively (2). In a Spanish study, EAEC was isolated from 14% of travelers with TD returning from developing countries (6). Our result, showing EAEC in 8% of TD patients, is in accordance with these previous reports.

We found a high number of subjects carrying *E. coli* nonsymptomatically. In many developing countries, this kind of carriage of EAEC or EPEC is common (16). Also, the high sensitivity of the PCR assay and the high infectious dose of these bacteria may explain the absence of symptoms in the subjects to whose normal microbial floras these bacteria do not belong. In this study, however, only cultivable diarrheal *E. coli* were identified, since PCR was performed from the growth on primary culture plates, not directly from stool samples. Interestingly, STEC strains were isolated from stools of one nonsymptomatic subject before and after the trip, although STEC and its nonsymptomatic carriage were uncommon in Finland in 1996 (13).

Causes of TD are numerous, but bacteria, and especially ETEC, are believed to be its major cause. The development of new molecular diagnostic methods has made it possible to reevaluate this issue. These methods have also made it possible to extend the analysis to new *E. coli* pathogroups, such as EAEC and STEC, as well as to the old EPEC group. This study, investigating fecal samples from subjects with and without TD, showed that EAEC and EPEC were the major organisms found in both groups of subjects. STEC and ETEC seemed to play limited roles in TD. Moreover, ETEC, which was not the major TD pathogen in this study, was detected in travelers only after a stay in South America. This study also showed that travelers are commonly exposed to multiple bacterial pathogens, of which some, especially *S. enterica*, are more common than *E. coli* in TD.

ACKNOWLEDGMENTS

We thank Tarja Heiskanen and Seija-Liisa Kyrönseppä for excellently taking, culturing, and storing the samples during the trip under rough conditions, and we thank Liisa Immonen, Marjut Saari, and Ritva Taipalinen for their technical assistance in the laboratory. Hannu Kyrönseppä is gratefully acknowledged for parasitological studies.

This work was financially supported by the Research Foundation of Orion Corporation.

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